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4. PERFORMING ORGANIZATION REPORT NUMBER(S)					5. MONITORING ORGANIZATION REPORT NUMBER(S)				
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60. NAME OF PERFORMING ORGANIZATION				Sb. OFFICE SYMBOL	7a. NAME OF MONITORING ORGANIZATION U. S. Army Research Office				
Stanford University				(If applicable) N/A					
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Stanford	, CA 9430	5			Research Triangle Park, NC 27709-2211				
8a. NAME OF ORGANIZA	FUNDING/SPO	NSORII	NG	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER				
	rmy Resea	rch (Office	(if applicative)	DAA629-85-K-0242				
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21. ABSTRACT SECURITY CLASSIFICATION

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FINAL TECHNICAL REPORT J.W. Frost/ R.N. Zare DAAG29-85-K-0242

Our goal for the overall research project was to obtain insight into the mechanism used by microbes to cleave the carbon to phosphorus (C-P) bond of organophosphonates. Escherichia coli whole cells and cell lysate were first examined by P NMR which did not reveal any degradation products. However, subsequent analysis of volatiles produced in sealed growths of E. coli cultured in medium where alkylphosphonic acids were the only source of phosphorus led to the discovery of hydrocarbons (Cordeiro, M.L.; Pompliano, D.L.; Frost, J.W. J. Am. Chem. Soc. 1986, 108, 332). Analysis of the volatiles relied on gas chromatographic separation and flame ionization detection.

With the discovery of methane formation when \underline{E} . \underline{coli} were grown on medium having methylphosphonic acid as the only phosphorus source, all of our hypotheses were invalidated as to how mechanistically the microbes were cleaving the organophosphonate C-P bond. Our efforts were further complicated by the lack of C-P cleavage activity in cell-free lysate of \underline{E} . \underline{coli} . Circumvention of these impediments has exploited chemical modeling, identification of products and intermediate metabolites involved in the biodegradation, and molecular biological analysis.

Carbon fragments produced by <u>E</u>. <u>coli</u> degradation of a wide range of alkyl- and alkenylphosphonates have been compared with the carbon fragments produced by various chemical methods developed for cleaving organophosphonate C-P bonds.(Frost, J.W.; Loo, S.; Cordeiro, M.L.; Li, D. <u>J. Am. Chem. Soc.</u> 1987, <u>109</u>, 2166). Correspondence of the products produced by the chemical degradation to those produced by the biotic degradation led to the proposal for a radical-based dephosphorylation process during organophosphonate C-P bond cleavage.

Two types of radical-based dephosphorylation processes can be One formulation involves intermediacy of a phosphoranyl envisioned. while the other proceeds through a phosphonyl radical. (A) Differentiation between these mechanistic hypotheses requires knowledge as to the fate of the phosphorus portion of organophosphonates immediately before and after cleavage of the C-P bond. To accomplish derivatization techniques suitable for laser-induced detection developed. Derivatization fluorescence were alkylphosphonic acids with p-(9-anthroyloxy)phenacyl bromide provided nearly quantitative yields of dipanacyl organophosphonates. detection limit for these derivatized organophosphonates was 20 fmol using 8 mW of 325-nm radiation from a He-Cd laser (Roach, M.C.; Ungar, L.W.; Zare, R.N.; Reimer, L.M.; Pompliano, D.L.; Frost, J.W. Anal. Chem. Unfortunately, the derivatization reagent did not 1987, <u>59</u>, 1056). esterify phosphoric acid or phosphorous acid which are two possible phosphorus-containing degradation products. Resolution of this problem required increasing the reactivity of the derivatization reagent.

have since discovered that a phenacyl group containing a triflate was reactive enough to esterify phosphoric acid, phosphorous acid, ethylphosphonic acid, and ethylphosphonous acid. Simultaneous with the laser-based detection system development, we have been optimizing methods for introduction of $^{32}\mathrm{P}$ into organophosphonates. This latter methodology is intended for capillary zone electrophoretic separation of metabolites interfaced with β emission detection.

The final area of activity where significant progress has been made involves molecular biological characterization of microbial C-P bond During analysis of aminomethylphosphonate biodegradation by cleavage. <u>E</u>. coli, carbon fragments were generated which suggested biodegradative mechanism similar to alkylphosphonate C-P bond cleavage. E. coli were then subjected to transposon mutagenesis. Mutants unable to degrade ethylphosphonate were appraised for ability to degrade aminomethylphosphonates. One mutant, E. coli SL724, was unable to degrade both alkyl- and aminomethylphosphonates. This mutant thus establishes a linkage at the genetic level between biodegradation of these two very different classes of organophosphonates (Avila, L.Z.: Loo, S.H.; Frost, J.W. <u>J. Am. Chem. Soc.</u> 1987, <u>109</u>, 6758). The gene which complements the mutation of SL724 has recently been cloned and used to establish the genomic location of the locus responsible for organophosphonate C-P bond cleavage (Loo, S.H.; Peters, N.K.; Frost, J.W. <u>Biochem</u>, <u>Biophys</u>, <u>Res</u>, <u>Commun</u>, 1987, <u>148</u>, 148).

PUBLICATIONS 22924-LS

M.L. Cordeiro, D.L. Pompliano, and J.W. Frost
 "Degradation and Detoxification of Organophosphonates: Cleavage
 of the Carbon to Phosphorus Bond", J. Am. Chem. Soc. <u>108</u>, 332. (1986).

- 2. J. W. Frost, S. Loo, M.L. Cordeiro, and D. Li "Radical-Based Dephosphorylation and Organophosphonate Biodegradation," J. Am. Chem. Soc. 1987, 109, 2166.
- 3. S.H. Loo, N.K. Peters, and J.W. Frost "Genetic Characterization of an Escherichia Coli Mutant Deficient in Organophosphonate Biodegradation," Biochem. Biophys. Res. Comm. 1987, 148.
- 4. L.Z. Avila, S.H. Loo, and J.W. Frost "Chemical and Mutagenic Analysis of Aminomethylphosphonate Biodegradation," J. Am. Chem. Soc. 1989, 109, 6758.
- 5. Mark C. Roach, Richard N. Zare, Leah M. Reimer and John W. Frost, "p-(9-Anthroyloxy) Phenacyl Bromide as a Fluorescence Derivatization Reagent for Organic Acidic Compounds in High performance Liquid Chromatography,". J. Chromatog., Submitted
- 6. L.M. Reimer, J.W. Frost, M.C. Roach, and R.N. Zare, "In Vivo Enzymatic Studies Using HPLC with Laser Induced Fluorescence Detection: Shikimate Buildup in Plants under Herbicidal Stress," J. Chromatog., Submitted.
- 7. J.W. Frost, Richard N. Zare, "Organophosphonate Biodegradation: Determining the Fate of Phosphorus," Submitted.

SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT

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- Leah M. Reimer- Graduate Research Assistant Ph.D. Nov. 88
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